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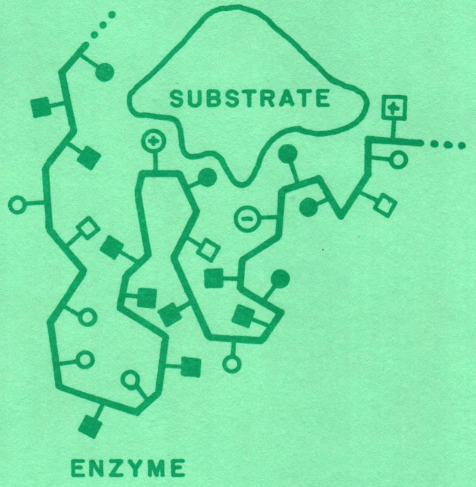
RESOURCE MANUAL

PH

THE PH SPECIFICITY OF ENZYMES



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HUNTINGTON TWO COMPUTER PROJECT

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PH

RESOURCE MANUAL

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PH

RESOURCE MANUAL

INTRODUCTION

Some of the material in this manual is written with the student, rather than the teacher, in mind. It is our hope that an interested student will be able to research and find his own answers to questions posed in the *PH* program, using certain materials in this manual as the necessary background.

Other parts of this manual are clearly meant for the teacher. Those sections that are intended solely for background reading will be marked with a ★, and the sections containing extended materials for the teacher will be marked with a ●. Sections marked with both (★●) indicate a mixed function.

I. BACKGROUND INFORMATION

★ Enzymes as Proteins

Structurally, enzymes are *proteins*; therefore, it is necessary to understand the nature of proteins in order to understand enzymes. Proteins are polymeric. A polymer may be thought of as a group of *similar* units bonded together in a chain. Figure 1 can be used to represent a polymer. In this case, the similar polymer unit is the circle. Notice that not all the circles are exactly the same. The definition of a polymer requires only that the units be similar.

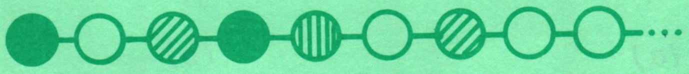


FIGURE I

We will take the "..." on the end of the chain to mean "and so on." This is another characteristic of most polymers: they are long. Also, the units that make up the polymer are usually bonded together in a particular fashion. We have now stated three requirements of proteins as polymers: they must be made up of similar units in a chain; the chain must be long; and the units should be bonded together in a particular fashion.

The units that make up proteins are called amino acids. All amino acids are structurally similar. Figure 2 below illustrates the basic structure of an amino acid. Each amino acid is based on a central carbon atom (1) to which four different groups are attached: the amino group (2) composed of a nitrogen and two hydrogens, the carboxyl or carboxyllic acid group (3), a single hydrogen (4) and a side group or replacement group (5). Of these groups, only the side group is allowed to vary from one amino acid to another.

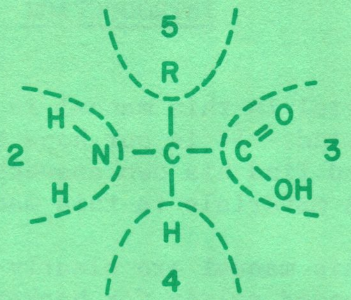
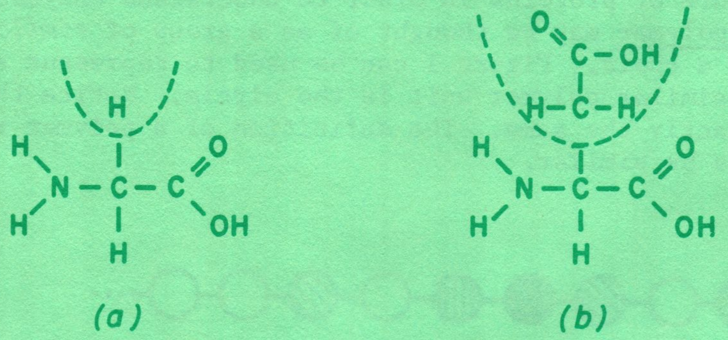


FIGURE 2

In Figure 3 you see two examples of amino acids. In glycine (a) the side group (R) is a single hydrogen atom; in aspartic acid (b) the side group is more complex. Both are amino acids, however, and can be found in protein polymers. There are over 20 other types of amino acids that can be found in proteins. Each of these amino acids (with two exceptions) is structurally identical except for the side (R) group.



(a) *(b)*

FIGURE 3

How long are protein polymers? To get some idea of what "long" means in the case of proteins, let's look at two examples: *insulin* and *collagen* Insulin is one of the shortest protein polymers known and has 51 amino acids arranged in two chains. Collagen, which is found in your hair and fingernails, can have as many as 3,000 amino acids -- and collagen is nowhere near the largest protein.

Proteins have their amino acid units bonded together by peptide bonds. Figure 4 shows the location of a peptide bond. The covalent bond is always between the amino group of one amino acid and the carboxyl group of the other.

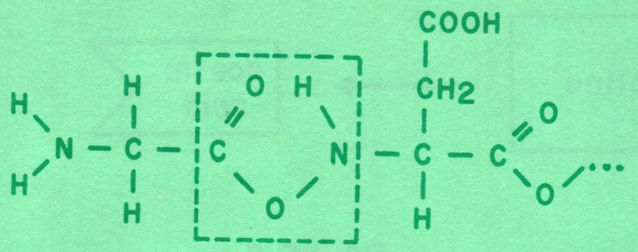


FIGURE 4

The free carboxyl groups can in turn be bonded to the amino group of another amino acid and so on until long chains result.

Proteins are not straight chains; they are folded over and about themselves. Studies have shown that this folding becomes an important consideration when one attempts to determine the properties of a protein. Heat and strong acids and bases are among the many factors that can change the folding of a protein. Think of the change that occurs when you cook egg white. Egg white is a solution of albumin, a protein.

★ Enzymes as Catalysts

We can picture a chemical reaction as a hill. No matter what the grade on the other side of the hill, it is going to take energy to get up to the crest of the hill. If we could in some way lower the top of the hill, with a bulldozer for instance, it would require less energy to get to the other side. Catalysts act by "lowering the hill."

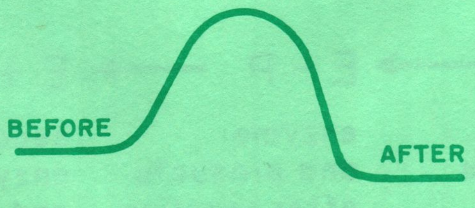


FIGURE 5

For an example, let's take an enzyme called acetylcholinesterase. This enzyme speeds the breakdown of the chemical acetylocholine into two parts, choline and acetic acid.

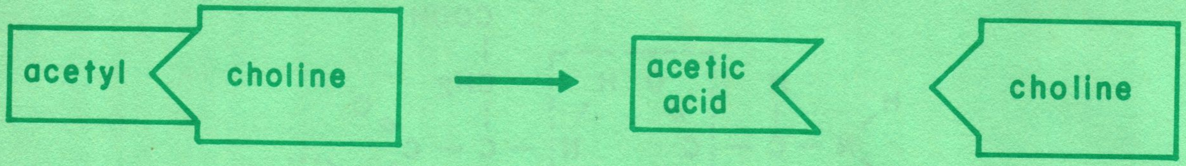


FIGURE 6

This break-up would occur naturally, but at a very slow rate, because the energy required to "get up the hill" is great. (This amount of energy is called the *activation energy* of the reaction.) In fact, the reaction would proceed so slowly that you would die because your nerves would stop functioning. How can we lower the activation energy so that the reaction will proceed more quickly?

To reduce the activation energy, we require a particular enzyme, since each enzyme is specific and is a catalyst for only one of a few reactions. An enzyme cannot lower the activation energy of any of the other reactions that might be going on at the same time. In our case, we require the enzyme acetylcholinesterase.

The enzyme first bonds to the substrate (the substance that the enzyme works on), forming an enzyme-substrate. Most enzymes are thought to have electrically charged groups that in some way lower the amount of energy required for the reaction. When the reaction is over, the enzyme releases the product.

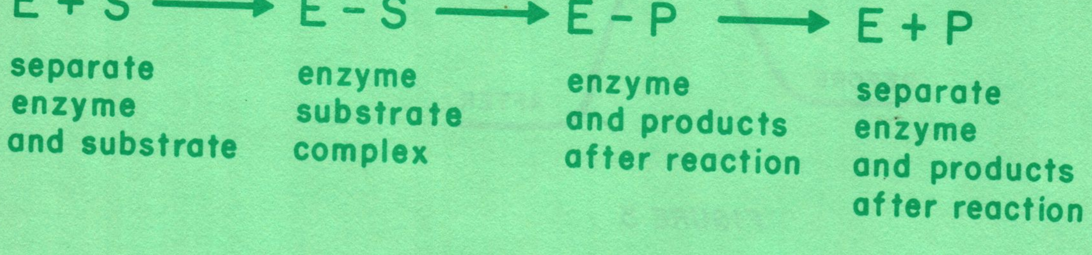

E + S
separate enzyme and substrate
E - S
enzyme substrate complex
E - P
enzyme and products after reaction
E + P
separate enzyme and products after reaction

FIGURE 7

Figure 7 shows the enzyme emerging from the reaction unchanged. This is another characteristic of a catalyst, that it takes part in a reaction but is itself unaltered by the reaction. For this reason, a catalyst (in our case, the enzyme) can be used over and over again. Furthermore, only a small amount of catalyst is required for a reaction involving a large amount of the substrate.

It is possible to depict a reaction in which an enzyme is present as a catalyst in the following way:

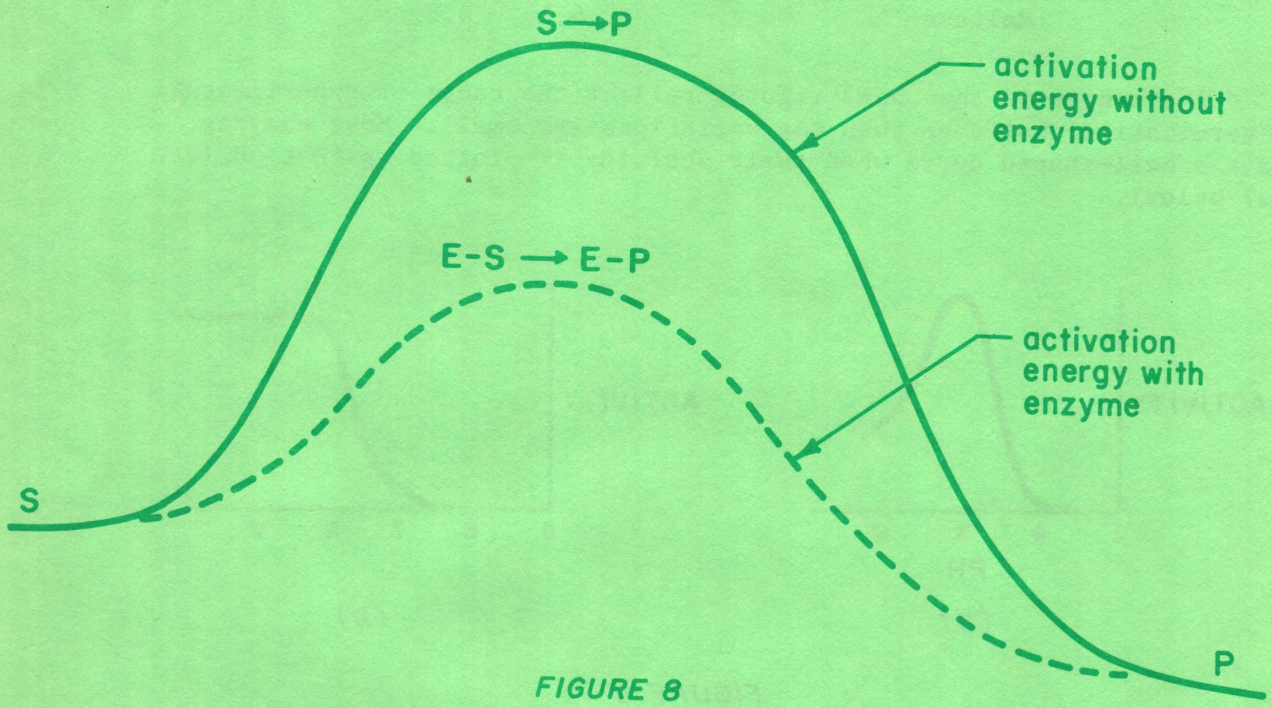


FIGURE 8

The enzyme does not change the substrate or the products that would normally form; it only lowers the activation energy, allowing the reaction to occur more easily and usually more rapidly.

★ Active Site of an Enzyme

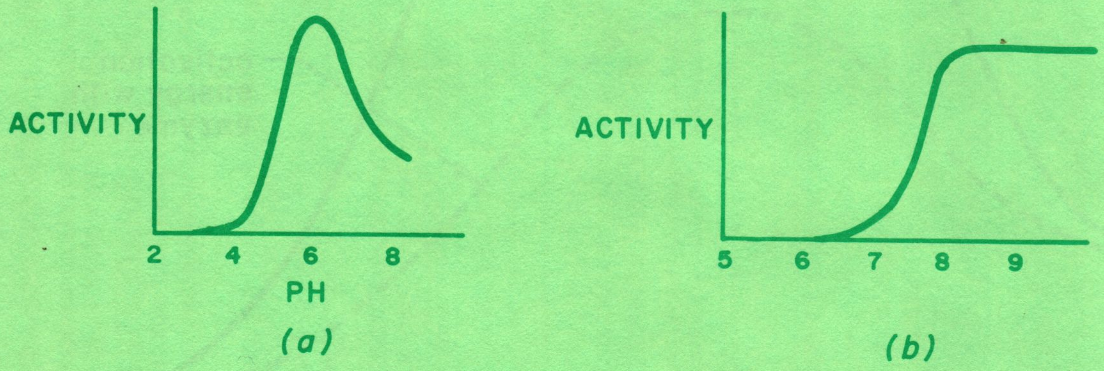
Once scientists discovered some of the mechanisms of enzyme action, they tried to answer another question: Does the whole enzyme take part in catalysis? Careful investigations using many different techniques have indicated that certain areas of an enzyme are very important for catalysis, while other areas have no effect on the enzyme's catalytic ability. This leads to the idea of an active site, the area of the enzyme on which catalysis is actually carried out. Many enzymes may have more than one active site, but all the active sites in such cases are involved in the catalysis of the same reaction.

★● On the pH Specificity of Enzymes

Nearly all enzymes exhibit pH optima. Some specific examples that you may find useful in class discussion are listed below:

Enzyme	pH Optimum
Salivary amylase	5.5 - 6.5
Pepsin	1 - 2
Pancreatic amylase	6.7 - 7.2
Trysin	7.8 - 8.7
Lipase	8 -
Maltase	6.1 - 6.8

The ranges in the above figures reflect the range of experimental determination; but even then the variations are small. Most enzymes have a bell-shaped curve when their activity is plotted against pH (see (a) below).



(a) *(b)*

FIGURE 9

However there are a few enzymes such as leucine aminopeptidase that show quite a different curve (see (b)). Why should enzymes in general show a bell-shaped curve and have restricted pH optima? And why do certain enzymes behave differently?

As we said before, the answers to these questions can become highly mathematical; while we do not expect high school students to understand the mathematical solutions involved, we hope that by using *PH* they will acquire a basic understanding of pH specificity -- the norm and the exceptions.

pH can affect (1) the affinity of an enzyme for its substrate; (2) the stability of the enzyme; or (3) the catalytic action of the enzyme. The effects of (1) and (2) can often be screened out of experimental results. When this is done the "bell-shaped curve" still remains. It seems, then, that a pH optimum is due primarily to the effect of different pH values on the catalytic action of the enzyme.

In general enzymes are thought to function through acid-base catalysis; in order for this type of catalysis to occur, there must be charged groups at the active site of the enzyme. Since enzymes are protein structures, the only possible charged groups will be amino acids. Luckily, there are only a few amino acids that can carry a charge on their side groups (R groups).

Those amino acids that can carry a positive charge on their side group (by picking up an additional hydrogen ion) are called basic amino acids. One example of a basic amino acid is lysine:

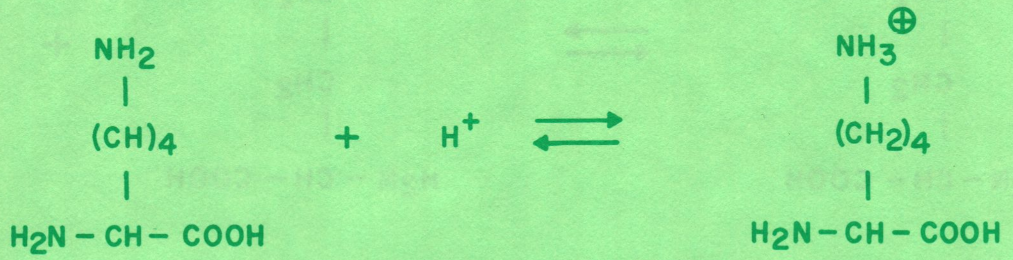
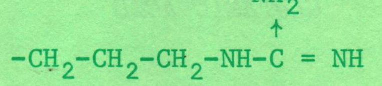


FIGURE 10

Since a hydrogen ion (H^+) is involved in such a reaction, a basic amino acid can carry a net positive charge only at certain pH values. While the pattern is the same for each of the basic amino acids, each basic amino acid has its own range of pH values in which it is charged.

BASIC AMINO ACIDS

pH at which the group is one-half ionized = pK_a

Name	pK_a	R-Group Structure
ARGININE	1.48	 $-CH_2-CH_2-CH_2-NH-\overset{\overset{NH_2}{\uparrow}}{C} = NH$
HISTIDINE	6.8	$-CH_2-C_3N_2H_3$
LYSINE	10.0	$-CH_2-CH_2-CH_2-CH_2-NH_2$

Other amino acids can carry a net negative charge (by releasing a hydrogen ion). These amino acids are called acidic. *Glutamic acid* is one example:

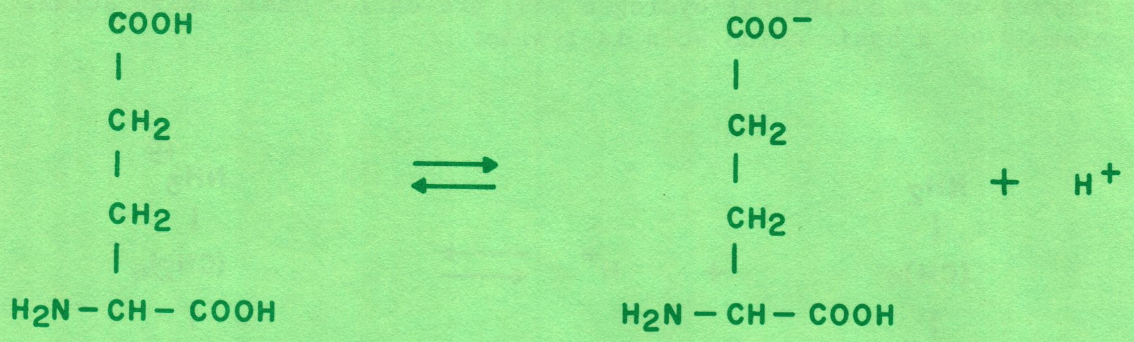


FIGURE 11

Likewise, each acidic amino acid has a certain particular range of pH values in which it is charged. For example, *glutamic acid* is highly charged at pH higher than 5, while *tyrosine* is charged only at pH higher than 11.

ACIDIC AMINO ACIDS

Name	pK_a	R-Group Structure
ASPARTIC ACID	4.6	$-CH_2-COOH$
CYSTEINE	8.3	$-CH_2-SH$
GLUTAMIC ACID	4.25	$-CH_2-CH_2-COOH$
TYROSINE	10.7	$-CH_2-C_6H_4-OH$

In general, basic amino acids are charged at low (acidic) pH values, while acidic amino acids are charged at high (basic) pH values. Your students should be able to develop this understanding through experimentation with the program *PH*.

★• Relation of Enzyme Activity to Amino Acid Charge

If a basic and an acidic amino acid are both present at the active site of an enzyme, a bell-shaped curve may develop. Since the basic amino acid is charged only at low pH values and the acidic amino acid is charged only at high pH values, they both will be charged only in the middle of the pH scale, if at all. If both the acidic and the basic amino acids are required for the enzyme's catalytic activity, then we will find that the enzyme has a restricted pH optimum somewhere around 7 on the pH scale.

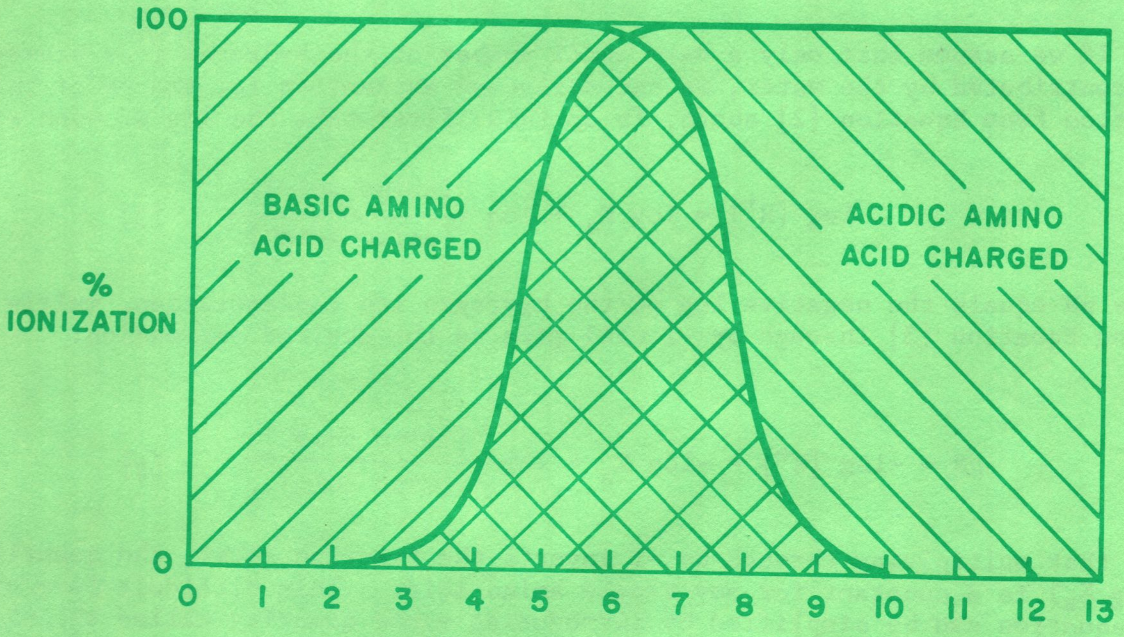


FIGURE 12

This would account for the bell-shaped curves seen for many enzymes. If the presence of both an acidic and a basic amino acid is necessary for the enzyme to function, we should expect to see catalytic activity only when both of them are in a charged state.

How does this explain the activity curve of an enzyme such as leucine aminopeptidase that has a uniform pH optimum over the entire basic range? By looking back at the last graph, you should be able to formulate a hypothesis.

• pH and Amino Acid Charge State

As for many other chemical reactions, a change in the charge of an amino acid can be described by an equilibrium equation. For the reaction

$$\text{amino acid} \rightleftharpoons H^+ + \text{amino acid}^-$$

we can write the following equilibrium expression:

$$K_a = \frac{[H^+]\,[\text{amino acid}^-]}{[\text{amino acid}]} \qquad [1]$$

Rearranging terms we obtain:

$$[H^+] = K_a \frac{[\text{amino acid}]}{[\text{amino acid}^-]} \qquad [2]$$

If we assume that only a very small number of the hydrogen ions present are contributed by the water, we can derive an expression for the pH of the solution from Equation [2] above. To do this, first take the log of each side:

$$\log\,[H^+] = \log K_a + \log \frac{[\text{amino acid}]}{[\text{amino acid}^-]} \qquad [3]$$

Since pH equals the negative log of the hydrogen ion concentration, multiplying Equation [3] through by -1 will give us the desired expression:

$$\text{pH} = -\log\,[H^+] = -\log K_a + \log \frac{[\text{amino acid}^-]}{[\text{amino acid}]} \qquad [4]$$

Note that multiplying through by -1 inverts the fraction. Also, the quantity ($-\log K_a$) is a constant for any single amino acid. This difficult form of the equation can be simplified by introducing a new quantity called the pK_a, the negative log of the constant K_a. By substituting this new quantity into Equation [4] and rearranging terms, we obtain:

$$\log \frac{[\text{amino acid}^-]}{[\text{amino acid}]} = \text{pH} - pK_a$$

Using this equation, it is possible to calculate the per cent of amino acid in the charged form at any pH. Although this equation was obtained using an acidic amino acid, a similar equation can be derived for basic amino acids. The program *PH* evaluates such equations for the student over the entire pH range from 0 to 14.

II. *PH* PROGRAM LISTING

```
100 REM PH -- PROGRAM TO DETERMINE PH VS. ENZYME ACTIVITY
110 REM COPYRIGHT 1973 - STATE UNIVERSITY OF NEW YORK
120 REM DEVELOPED BY J. FRIEDLAND
130 REM PROGRAMMED BY J. FRIEDLAND, L. KAUFMAN
135 REM REVISED BY B. ESRIG
140 REM LATEST REVISION: 10-2-73
150 DIM A(5),D(8)
160 FOR I=1 TO 7
170 READ D(I)
180 NEXT I
190 PRINT "DO YOU WISH INSTRUCTIONS (1=YES, 0=NO)";
200 INPUT Q
210 IF Q=0 THEN 250
220 LET N=0
230 IF Q<>1 THEN 190
235 PRINT
240 GOTO 300
250 PRINT "CODE LIST (1=YES,0=NO)";
260 INPUT N
270 IF N=0 THEN 615
280 IF N<>1 THEN 250
290 GOTO 440
300 PRINT "  THEORY HOLDS THAT THE BEHAVIOR OF ENZYMES IS IN PART"
310 PRINT "A FUNCTION OF THE TYPES OF AMINO ACIDS AT THE ACTIVE SITE."
320 PRINT "THIS PROGRAM ALLOWS YOU TO SET THE NUMBER AND TYPE OF"
330 PRINT "IONIZABLE AMINO ACID(S) PRESENT."
340 PRINT
350 PRINT "  THE COMPUTER WILL FIRST ASK:"
360 PRINT "'HOW MANY IONIZABLE AMINO ACIDS AT THE ACTIVE SITE?'"
370 PRINT "YOU CAN PICK ANY WHOLE NUMBER FROM 1 TO 4.  REAL ENZYMES"
380 PRINT "ARE THOUGHT TO HAVE AT LEAST TWO AMINO ACIDS"
390 PRINT "PRESENT, BUT TRY DIFFERENT NUMBERS."
400 PRINT
410 PRINT"   THEN THE COMPUTER WILL ASK:"
420 PRINT" 'AMINO ACID 1  ---- CODE NO.?'"
430 PRINT "YOU CAN INPUT THE CODE NUMBER FOR ANY OF THE FOLLOWING ";
435 PRINT "AMINO ACIDS:"
440 PRINT "   CODE     NAME      R-GROUP OF AMINO ACID"
450 PRINT "   ----     ----      ---------------------"
460 PRINT "    1   ASPARTIC ACID   -CH2-COOH"
470 PRINT "    2   CYSTEINE        -CH2-SH"
480 PRINT "    3   GLUTAMIC ACID   -CH2-CH2-COOH"
490 PRINT "    4   TYROSINE        -CH2-C6H4-OH"
500 PRINT "                                       NH2"
510 PRINT "                                        ↑"
520 PRINT "    5   ARGININE        -CH2-CH2-CH2-NH-C=NH"
530 PRINT "    6   HISTIDINE       -CH2-C3N2H3"
540 PRINT "    7   LYSINE          -CH2-CH2-CH2-CH2-NH2"
```

```
550 PRINT
560 IF N=1 THEN 615
570 PRINT "AMINO ACIDS 1-4 ARE CALLED ACIDIC AMINO ACIDS"
580 PRINT "AMINO ACIDS 5-7 ARE BASIC"
590 PRINT "BY TRYING ACIDIC AND BASIC AMINO ACIDS SEPARATELY AND IN"
600 PRINT "COMBINATION YOU CAN DEVELOP TYPICAL PH PATTERNS ";
605 PRINT "FOR ENZYMES."
610 PRINT
615 PRINT
620 PRINT "HOW MANY IONIZABLE AMINO ACIDS AT ACTIVE SITE (UP TO 4)";
630 INPUT N
640 IF ABS(N-2.5)>1.5 THEN 620
650 IF N<>INT(N)THEN 620
660 PRINT
670 FOR T=1 TO N
680 PRINT "AMINO ACID";T;" ---- CODE NO.";
690 INPUT B
700 IF INT(B)<>B THEN 680
710 IF ABS(B-4)>3 THEN 680
720 LET C=1
730 PRINT " SHOULD IT BE CHARGED (1=YES, 0=NO)";
740 INPUT Q
750 IF Q=1 THEN 780
760 IF Q<>0 THEN 730
770 LET C=-1
780 LET A(T)=C*D(B)
790 NEXT T
800 PRINT
810 PRINT "PH RANGE FOR THIS EXPERIMENT:"
820 PRINT "  LOWER LIMIT";
830 INPUT P1
840 IF ABS(P1-7)<=7 THEN 870
850 GOSUB 1210
860 GOTO 820
870 LET P1=INT(P1*2)/2
880 PRINT "  UPPER LIMIT";
890 INPUT P2
900 IF P2<P1 THEN 880
910 IF P2<=14 THEN 940
920 GOSUB 1210
930 GOTO 880
940 LET P2=INT(P2*2+.999)/2
950 PRINT
960 PRINT "           RELATIVE ENZYME ACTIVITY"
970 PRINT"      0   10   20   30   40   50   60   70   80   90  100"
980 PRINT" PH   I----I----I----I----I----I----I----I----I----I----I"
990 FOR P=P1 TO P2 STEP .5
```

```
1000 LET Z=1
1010 FOR T= 1 TO N
1020 IF A(T)>0 THEN 1050
1030 LET L=P+A(T)
1040 GOTO 1060
1050 LET L=A(T)-P
1060 LET E=1/(10↑L+1)
1070 LET Z=Z*E
1080 NEXT T
1090 IF P<>INT(P) THEN 1110
1100 PRINT P;
1110 PRINT TAB(5);"I";TAB(INT(50*Z+6.5));"*"
1120 NEXT P
1130 PRINT
1140 PRINT "ANOTHER EXPERIMENT (1=YES, 0=NO)";
1150 INPUT Q
1160 IF Q=1 THEN 610
1170 IF Q<>0 THEN 1140
1180 STOP
1210 PRINT
1220 PRINT "PH RANGE IS LIMITED TO FROM 0 TO 14"
1230 PRINT
1240 RETURN
1250 DATA 4.6,8.3,4.25,10.97,-1.48,-6.8,-10.0
1260 END
```

III. THE *PH* MODEL

Program Equations and Assumptions

1) Equations derived from the Henderson-Hasselbach equation for weak acids are assumed to be a valid description for the reactions of side groups of amino acids:

$$\log(\text{ionized/de-ionized}) = \text{pH} - \text{p}K_a \quad \text{for acidic groups}$$

$$\log(\text{ionized/de-ionized}) = \text{p}K_a - \text{pH} \quad \text{for basic groups}$$

2) When more than one ionizable amino acid is present at the active site, all amino acid side groups are assumed to be equally important in carrying out catalysis:

$$\begin{aligned}\text{catalytic ability} &= (\%\ \text{amino acid \#1 with proper charge})\\ &\times (\%\ \text{amino acid \#2 with proper charge})\\ &\times \ldots\ldots\end{aligned}$$

3) None of the amino acids at the active site are N-terminal or C-terminal; thus alpha amino and alpha carboxyl groups are not significant.

4) Neither the structure of the enzyme nor the structure of the substrate is assumed to have a modifying effect on catalysis.

5) pH is assumed to affect only the enzyme, not the substrate.

6) At no time does the enzyme become irreversibly denatured.

Program Variables and Their Meaning

A(T)	pK_a of amino acid #T multiplied by identifier (+1 for acidic, -1 for basic amino acids)
B	Amino acid code number holder
C	Charged state identifier (-1 means uncharged)
D(T)	pK_a holder
E	Per cent of amino acid in correctly charged state
N	Number of amino acids at active site
P	pH
P1	Lower limit of pH determination
P2	Upper limit of pH determination
Q	Response holder
T	Counter
Z	Catalytic ability of the enzyme

IV. BIBLIOGRAPHY

Sources for Additional Background on *PH*

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